

The Impact of Chronic Pelvic Ischemia on LUTS and Urinary Levels of Neuroinflammatory, Inflammatory, and Oxidative Stress Markers in Elderly Men: A Case-control Study



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| OBJECTIVE | To investigate lower urinary tract symptoms (LUTS) and urinary levels of neuroinflammatory, inflammatory, and oxidative stress markers in elderly men with chronic pelvic ischemia (CPI) caused by significant aortoiliac disease. |
| MATERIALS AND METHODS | Thirteen men aged over 60 years, with aorta, unilateral or bilateral common/internal iliac artery occlusion documented by computed tomography angiography or angiography, were enrolled from the vascular surgery department. Twelve sex- and age-matched controls without significant aortoiliac disease were used for comparison. Exclusion criteria included neurogenic bladder dysfunction, bladder or prostate cancer, prostatic surgery, pelvic radiotherapy, or chronic treatment for LUTS. Participants underwent urological examination, including assessment of International Prostate Symptom Score (IPSS), uroflowmetry, postvoid residual (PVR), and prostate volume. Urine samples were collected, and levels of neuroinflammatory (nerve growth factor, NGF), inflammatory (cytokines), and oxidative stress markers (8-hydroxy-2'-deoxyguanosine) were determined by enzyme-linked immunosorbent assay. |
| RESULTS | Groups were similar for age, PVR, prostate volume, and most cardiovascular risk factors. IPSS was higher in patients with CPI (11 ± 3 vs 8 ± 2 , $P = .02$), with a significant mean difference between groups of three points. Urinary NGF was significantly higher in men with CPI (3.7 ± 0.8 vs 2.9 ± 0.7 , $P = .02$), but no differences were found in inflammatory and oxidative biomarkers among groups. |
| CONCLUSION | Severe CPI in elderly men is associated with a significant increase in LUTS and bladder neurogenic inflammation, as suggested by the increase of NGF release in urine, sensitizing bladder afferents. These findings confirm the relevance of ischemia in bladder function and appear to validate animal models of bilateral iliac artery occlusion. UROLOGY 123: 230–234, 2019. © 2018 Elsevier Inc. |

Increasing evidence suggests that atherosclerosis and subsequent chronic pelvic ischemia (CPI) affecting

the bladder may be a cause of lower urinary tract symptoms (LUTS) in advancing age.¹

Several experimental models using rabbits and rats have demonstrated that CPI leads to bladder ischemia. It is well known that prolonged bladder ischemia induces morphologic and functional changes in bladder innervation, urothelium, detrusor muscle, and the endothelium of microvessels.² Interestingly, studies with these models indicate that the severity and duration of bladder ischemia are relevant to the type of bladder dysfunction. In other words, moderate ischemia is associated with bladder hyperactivity, whereas severe and long-term ischemia would result in bladder underactivity.³⁻⁵

Atherosclerosis is very common in elderly age, especially in the abdominal aorta and iliac arteries, specifically

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at bifurcations, hence reducing pelvic blood flow and leading to chronic bladder ischemia.² In addition, advanced age is strongly associated with functional, cellular, and molecular changes in vasculature, causing morphologic and functional changes in the bladder.⁵ Aging-related endothelial dysfunction is associated with a reduction of nitric oxide bioavailability, oxidative stress and consequent dysfunctional production of vasoconstrictor and vasodilator factors, prolonged low-grade inflammation, impaired angiogenesis, and endothelial cell senescence.⁵ All these cellular changes are believed to contribute to the increased prevalence of LUTS among elderly subjects. Moreover, the fact that α 1-blockers, the mainstay therapy for male LUTS, can improve bladder blood flow, promote smooth muscle relaxation, and reduce afferent signaling and oxidative stress in the context of ischemia further supports the hypothesis of vascular dysfunction in the etiopathogenesis of LUTS.^{5,6} Interestingly, there are also a large number of clinical studies reporting a significant association between aging, cardiovascular risk factors, erectile dysfunction, and the prevalence of LUTS.⁶

Despite above-mentioned associations, a direct impact of CPI on the appearance of LUTS in elderly men was never fully determined. Herewith, we investigated the occurrence of LUTS and the urinary levels of neuroinflammatory, inflammatory, and oxidative stress markers in elderly men with CPI caused by aorta, unilateral or bilateral common/internal iliac obstruction. A group of age-matched men without CPI were used as controls.

MATERIALS AND METHODS

The study was approved by the local ethics committee. Informed consent was obtained from all participants.

A case-control study was conducted. Cases featured 13 men aged over 60 years with aortic, unilateral or bilateral common/internal iliac artery occlusion, documented by computed tomography) angiography or conventional angiography, enrolled from the vascular surgery department, between October 2015 and December 2016. Twelve sex- and age-matched controls, without significant aortoiliac disease confirmed by abdominal aortoiliac duplex ultrasonography, were used for comparison. Ankle-brachial index in the worst limb was determined for cases.

Exclusion criteria included neurogenic bladder dysfunction, bladder or prostate cancer, prostatic surgery, pelvic radiotherapy, chronic treatment for LUTS, and history of stroke.

Evaluation comprised anamnesis, including assessment of cardiovascular risk factors, pelvic examination, uroflowmetry with Qmax calculation, suprapubic ultrasound to measure postvoid residual volume, transrectal ultrasound to determine prostate volume, and assessment of LUTS severity by means of International Prostate Symptom Score (IPSS). IPSS classified the severity of the disease as mild (0-7), moderate (8-19), and severe symptomatic (20-35). Total IPSS and storage/voiding subscores were determined.

Urine Collection

Urine was collected in sterile containers when subjects experienced a sensation of full bladder at the outpatient clinic. Samples were placed on ice and centrifuged at 3000 rpm for 10 minutes at 4°C. Supernatant was collected in 1-mL aliquots, and the remainder was used to measure creatinine (Cr) concentration. Aliquots were frozen at -80°C until further processing.

Urine Analysis

Urine samples were thawed to measure 8-hydroxy-2'-deoxyguanosine (8-OHdG), inflammatory cytokines, and urinary nerve growth factor (NGF).

In order to evaluate the level of oxidative stress, the competitive quantity of 8-OHdG was determined by enzyme-linked immunosorbent assay (Abcam ab201734), following the manufacturer's protocol. Summarily, urine samples of the control and ischemic patients and standards were added to an 8-OHdG-coated 96-well plate that was detected with horseradish peroxidase conjugated 8-OHdG antibody. A colored signal was obtained after incubation with tetramethylbenzidine substrate, and the absorbance was measured at 450 nm, using a multiplate reader (Tecan infinite M200, Männedorf, Switzerland). The average absorbance value of samples was plotted on the standard curve to extrapolate sample concentrations. Final values were then normalized to Cr concentration (8-OHdG/Cr, ng/mg).

Inflammation was measured through quantification of 40 different cytokines, specifically Eotaxin, Eotaxin-2, GCSF, GM-CSF, ICAM-1, IFN-gamma, I-309, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-6, IL-6sR, IL-7, IL-8, IL-10, IL-11, IL-12p40, IL-12p70, IL-13, IL-15, IL-16, IL-17, IP-10, MCP-1, MCP-2, M-CSF, MIG, MIP-1alpha, MIP-1beta, MIP-1delta, RANTES, TGF-beta1, TNF-alpha, TNF-beta, sTNF RI, sTNF-RII, PDGF-BB, and TIMP-2, using an ELISA kit (Abcam ab134003). Succinctly, the urine samples of the control and ischemic patients were incubated at 4°C overnight, with membranes having captured antibodies against the above-mentioned cytokines. Afterwards, membranes were incubated with biotinylated antibodies and streptavidin horseradish peroxidase. Signal was obtained through chemiluminescence using Chemidoc MP, and the signal intensity was analyzed using Image Lab 5.1.

To estimate the presence of chronic neurogenic inflammation, urinary NGF levels were measured by enzyme-linked immunosorbent assay Emax ImmunoAssay System (Promega, USA), following the manufacturer's instructions as used in other studies. Briefly, well plates are coated overnight with anti-NGF antibodies, which bind soluble NGF from urine samples. Captured NGF is recognized by a second specific antibody. After several washes, a horseradish peroxidase linked antibody is added, followed by incubation with a chromogenic substrate. The amount of NGF in the sample is proportional to the color generated by the enzymatic degradation of the substrate. The amount of NGF in the sample was measured at 450 nm using a Synergy HT microplate reader (BioTek Instruments, USA). All samples were run in duplicate and values were averaged against a standard curve generated with known amounts of NGF. Final values were then normalized to Cr concentration (NGF/Cr, pg/mg).

Statistical Analysis

All data are presented as mean \pm standard deviation.

The Kolmogorov-Smirnov test was used to check the normality of variable distributions. The distribution of NGF levels was log transformed to reduce skewness.

Unpaired Student *t* and Mann-Whitney *U* tests were used for statistical analysis between groups when considering parametric and nonparametric data, respectively.

Statistical significance was considered at *P* < .05. GraphPad Prism 7 statistics software for Mac was used.

RESULTS

Clinical and demographic characteristics of patients with CPI and controls are summarized in Table 1. Both groups were identical for age, prostate volume, postvoid residual volume, maximum urinary flow rate (Q_{max}), and most cardiovascular risk factors, namely hypertension, diabetes mellitus type 2, and dyslipidemia. In cases, mean ankle-brachial index in the worst limb was 0.42 ± 0.12. In controls, no obstructive disease was detected and triphasic flow was detected distally.

Mean total IPSS was significantly higher in the pelvic ischemia group than that in controls, with a between-group difference of three points (10.9 ± 3.5 vs 8.3 ± 2.2, *P* = .02). When comparing both groups for IPSS voiding and storage subscores, although a statistically significant difference between the two groups was not found, a trend for a higher mean voiding subscore occurred in men with CPI (7.6 ± 3.8 vs 5.4 ± 1.3, *P* = .08) (Fig. 1).

Urinary biomarkers of the ischemic and control groups are summarized in Table 2. Urinary NGF/Cr ratio was significantly higher in the ischemic group (3.7 ± 0.8 vs 2.9 ± 0.7, *P* = .02), but no differences were found in urinary cytokines and oxidative biomarkers. From the 40 human inflammatory cytokines investigated in the urine, MIP-1δ, sTNF RI, sTNF RII, ICAM-1, MCP-1, IL-6, and IP-10 presented slightly stronger signals in ischemic patients, albeit statistical significance was not obtained. The factors MIP-1β, TIMP-2, and IL-6 sR showed readable identical signals in both groups. All other 30 cytokines demonstrated weak or no signal. Considering oxidative stress

assessment, no significant difference was found in mean urinary levels of 8-OHdG between groups.

COMMENT

The most striking result to emerge from the present study is that the decrease of pelvic blood perfusion in elderly men is associated with a significant increase in LUTS and high urinary NGF levels.

Nonetheless, the above-mentioned symptoms were not severe as it could be expected from data extracted from experimental pelvic ischemia, but rather moderate according to the IPSS (mean IPSS in the pelvic ischemia group: 10.9 ± 3.5). In accordance, several studies evaluating the effect of internal iliac artery exclusion for patients undergoing endovascular aneurysm repair⁷ and patients with severe distal aortoiliac occlusive disease (Leriche syndrome)⁸ did not present a significant surge of urinary symptoms, but instead a high prevalence of erectile dysfunction, buttock claudication, and decreased distal pulses. In addition, it has been documented that the

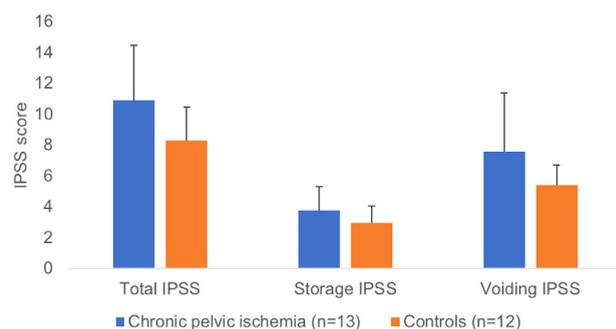


Fig. 1. Total IPSS and storage/voiding subscores in patients with chronic pelvic ischemia and controls. Mean total IPSS was significantly higher in the pelvic ischemia group than that in controls (10.9 ± 3.5 vs 8.3 ± 2.2, *P* = .02). No significant differences were found for IPSS voiding and storage subscores. Statistical significance was considered at *P* < .05. (Color version available online.)

Table 1. Clinical and demographic characteristics of patients with chronic pelvic ischemia and controls. Data of continuous variables are expressed as mean ± standard deviation. Statistical significance was considered at *P* < .05.

| | Chronic Pelvic Ischemia (<i>n</i> = 13), Aortic, Unilateral or Bilateral Common/Internal Iliac Artery Occlusion | Controls (<i>n</i> = 12), No Significant Aortoiliac Disease | <i>P</i> |
|---------------------------------------|---|--|----------|
| Age | 69.7 ± 7.7 | 68.8 ± 8.7 | .75 |
| BMI (kg/m ²) | 24.6 ± 2.5 | 28.1 ± 4.0 | .02 |
| Hypertension (<i>n</i>) | 12 | 11 | .95 |
| Diabetes mellitus type 2 (<i>n</i>) | 4 | 5 | .57 |
| Dyslipidemia (<i>n</i>) | 11 | 10 | .93 |
| Prostate volume (mL) | 34.3 ± 15.5 | 34.9 ± 19.7 | .93 |
| IPSS (0-35) | 10.9 ± 3.5 | 8.3 ± 2.2 | .02 |
| IPSS storage (0-15) | 3.8 ± 1.5 | 2.9 ± 1.1 | .14 |
| IPSS voiding (0-20) | 7.6 ± 3.8 | 5.4 ± 1.3 | .08 |
| Q _{max} (mL/s) | 16.8 ± 6.7 | 18.6 ± 7.7 | .60 |
| Postvoid residual volume (mL) | 42.9 ± 78.3 | 34.9 ± 19.7 | .60 |

Table 2. Expression of urinary nerve growth factor (NGF), inflammatory cytokines and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in patients with chronic pelvic ischemia and controls. Urinary levels of NGF and 8-OHdG were normalized to urine creatinine (Cr). Data of continuous variables are expressed as mean \pm standard deviation. Statistical significance was considered at $P < .05$.

| | Chronic Pelvic Ischemia ($n = 13$), Aortic, Unilateral or Bilateral Common/Internal Iliac Artery Occlusion | Controls ($n = 12$), No Significant Aortoiliac Disease | <i>P</i> |
|----------------|--|---|----------|
| NGF/Cr | 3.7 \pm 0.8 | 2.9 \pm 0.7 | .02 |
| MIP-1 δ | 3609 \pm 4692 | 1980 \pm 1834 | .29 |
| MIP-1 β | 4547 \pm 2852 | 6051 \pm 4386 | .36 |
| sTNF RI | 11,269 \pm 9952 | 6536 \pm 4131 | .15 |
| sTNF RII | 33,223 \pm 19,967 | 24,584 \pm 11,746 | .24 |
| ICAM-1 | 5421 \pm 3212 | 3154 \pm 2200 | .08 |
| TIMP-2 | 18,423 \pm 22,262 | 19,036 \pm 19,384 | .95 |
| MCP-1 | 18,368 \pm 16,787 | 18,199 \pm 8426 | .98 |
| IL-6 | 1348 \pm 2754 | 873 \pm 527 | .59 |
| IL-6 sR | 17,502 \pm 9621 | 19,313 \pm 9799 | .65 |
| IP-10 | 9105 \pm 7510 | 11,570 \pm 4967 | .37 |
| 8-OHdG/Cr | 70.4 \pm 32.7 | 68.1 \pm 35.9 | .86 |

maintenance of perfusion to the pelvis and lower extremities in aortoiliac occlusive disease can be obtained through collateral pathways, specifically the superior rectal artery and rectal plexus, the internal thoracic artery, as well as the sacral plexus.⁹ It is therefore possible that our study population presented moderate LUTS due to the very slow development of arterial obstruction, which gives time to the appearance of compensatory collateral pathways, preventing critical bladder ischemia. Taking that into account, we postulate that, in a longer time span, older patients with significant peripheral atherosclerotic disease would more likely have the alternative pathways impaired, and consequently severe bladder ischemia.

Several experimental studies were recently carried out to investigate the effect of chronic bladder ischemia on bladder function.^{3, 10} Short-term ischemia, through activation of molecular cascades with cell survival signaling, upregulation of cytokines, and accumulation of inflammatory eicosanoids and leukotriens, results in overactivity.⁶ In particular, increased expression of NGF, prostaglandins, proinflammatory cytokines, HIF-1 α , transforming growth factor β , vascular endothelial growth factor, cytokine-inducible nitric oxide species, and decreased endothelial and neuronal nitric oxide species seem to take part in this response to ischemia in urothelial cells.^{6, 11} On the other hand, prolonged and severe ischemia, by exhausting smooth muscle energy resources, due to excessive metabolic demands for adaptation to the extreme levels of hypoxia, results in mitochondrial damage, smooth muscle atrophy, fibrosis, contractile dysfunction, as well as further neurodegeneration, progressing from overactivity to underactivity.^{4, 12}

In a recent study in rats, balloon-induced endothelial injury of the iliac arteries combined with a high-cholesterol diet induced arterial occlusive disease and bladder ischemia, with consequent detrusor overactivity.¹⁰ In a subsequent study by the same authors, chronic pelvic arterial occlusive disease plus vascular endothelial dysfunction produced severe vascular damage and ischemia, leading to

increased postvoid residual volume, impaired detrusor contractility, and bladder fibrosis. These findings suggest that in rats, progressive vascular damage causes bladder dysfunction, which develops from bladder hyperactivity to bladder underactivity.³ Nevertheless, it remains to be established whether this hypothesis also applies to humans. Considering this chronicity, we can hypothesize that the CPI patients in our study, with high levels of urinary NGF, might be in the early phase of ischemia, indicating that with a longer exposure to hypoxia a current compensation of bladder function through hyperactivity could progress to underactivity.

In the literature, it seems clear that an adequate perfusion can be maintained until at least 75% of the maximum bladder capacity is reached; after that limit, blood flow tends to decrease. Reperfusion occurs after bladder distention, with release of vasodilating cytokines. In an already ischemic bladder, especially in the presence of excessive chronic bladder filling, repeated ischemia-reperfusion cycles could additionally damage the bladder, by increasing oxidative stress, denervation, and expression of tissue-damaging molecules, like NGF and prostaglandins.^{5, 13}

Urothelium and suburothelial vessels, being the most active metabolic layers of the bladder, are in a greater risk of suffering from ischemia, and since most afferent nerves are in the lamina propria and are greatly sensitive to hypoxia, both motor and sensory innervations are impaired, resulting in bladder dysfunction.⁵ As the most affected layer by decreased perfusion, the urothelium might be the major source of urinary cytokines and neurotrophins. In animal models of CPI, a marked expression of NGF in the urothelium was observed.¹⁴ NGF is important for maintaining bladder afferent activity, and increased NGF expression may lead to sensitization of neural sensory pathways, contributing to bladder dysfunction, particularly overactivity.¹⁴ The low-grade inflammation associated with ischemia can explain the increase of urinary NGF in men with CPI, promoting the sensitization of

bladder primary afferents. These findings seem to validate animal models of chronic bladder ischemia used to investigate ischemia-induced bladder dysfunction.

Throughout the years, oxidative stress has been greatly associated to the pathophysiology of ischemia-induced bladder dysfunction. Using both rabbit and rat models, it has been demonstrated that pelvic arterial insufficiency leads to bladder ischemia and oxidative stress linked to the upregulation of oxidative stress-sensitive genes. In this context, a pro-oxidative environment provokes contraction of detrusor muscle and bladder overactivity, which decreases subsequently after administration of antioxidant therapy.^{5, 6} The mechanism by which oxidative stress causes bladder dysfunction seems to be through sensitization of afferent pathways, as shown in animal models that had increased tachykinin-containing nerves and upregulation of neurokinin receptors.¹³ It has been further attested that increasing age resulted in higher amounts of reactive oxygen species, O₂⁻, and 8-OHdG in the urothelium of mice.⁶ Our ischemic patients presented minor oxidative stress levels, most likely by means of abundant microvascular collateral pathways, thus being unable to fully replicate the above-mentioned animal models.

This study has some other obvious limitations. Sample size was small, and the study is restricted to one center. Therefore, validation of these results should be considered with some caution. Moreover, urine sample storage and the enzyme-linked immunosorbent assay methodology for measuring urinary NGF, cytokines, and oxidative stress markers are not yet standardized.

CONCLUSION

In conclusion, severe pelvic ischemia in elderly men is associated with a significant increase in LUTS and bladder neurogenic inflammation, as suggested by the increase of NGF release, which may sensitize bladder primary afferents. These findings confirm the relevance of CPI in bladder function and validate animal models of bilateral iliac artery occlusion currently under use to investigate the pathophysiologic mechanisms at stake. However, the chronic evolution of the disease in men, when compared with the sudden arterial obstruction induced in experimen-

tal models, permits the development of collateral circulation, limiting the direct translation of the animal data.

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